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GC-MS ANALYSIS OF BIOACTIVE COMPONENTS OF *ARISTOLOCHIA KRYSAGATHRA* (ARISTOLOCHIACEAE)

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ABSTRACT

Aristolochia krysagathra belongs to the family Aristolochiaceae is well known in Indian traditional system for its traditional uses. The present investigation was carried out to determine the possible bioactive components of whole plant of *Aristolochia krysagathra* using GC-MS analysis. Thirteen compounds were identified from the whole plant of *Aristolochia krysagathra.* The prevailing compounds in the ethanol extract of whole plant of *Aristolochia krysagathra* were 3-O-Methyl-d-glucose (43.18%), 9, 12, 15-Octadecatrienric acid, methyl ester, (Z, Z, Z)-(11.46%), n-Hexadecanoic acid (10.25%), β-Sitosterol (9.53%), 3, 7, 11, 15-Tetramethyl-2-hexadecan-1-ol (7.12%), Stigmasterol (4.83%), Phytol (4.46%), 9, 12, Octadecadienoicacid (Z, Z) (4.34%) and Octadecanoic acid (2.17%).

Key words: *Aristolochia krysagathra*, GC-MS, Phytol, Stigmasterol.

INTRODUCTION

Phytochemicals are present in a variety of plants, and are utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables[1]. Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "Secondary metabolites"[2]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate[3]. There is growing awareness in correlating the phytochemicals constituents of a medicinal plant with its pharmacological activity[4-7]. Screening of active compounds from plants has lead to the invention of new medicinal drugs, which have efficient protection and treatment roles against various diseases including cancer and Alzheimer's diseases[8,9].

The genus *Aristolochia* finds a prominent place in different Indian systems of medicine. The different ethnic communities in India have used different species of *Aristolochia* in the treatment of various human ailments[10-12]. Kanikkar tribals of Kalakad-Mundanthurai Tiger Reserve Sanctuary, Tamil Nadu, boiled the equal quantity of fresh root and leaves of *Aristolochia krysagathra* in coconut oil for about

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15-20 min. over a low flame. The oil is filtered after cooling and applied on the head once in a day as the treatment of rheumatism. The therapy is used to reduce excessive heat of the body[12]. Taking into consideration of the medicinal importance of this plant, the ethanol extract of whole plant of *Aristolochia krysagathra* were analyzed for the first time using GC-MS. This work will help to identify the compounds of therapeutic value.

EXPERIMENTAL

Materials and methods

The whole plant of *Aristolochia krysagathra* Sivaranjan and Pradeep were collected from the natural forest of Kalakad-Mundanthurai Tiger Reserve Forest, Western Ghats, Tirunelveli, Tamil Nadu, India. The plant was identified with help of local flora and authenticated in Government of India, Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu.

GC-MS analysis

GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30 mm x 0.25 mm 1 D x 1 μMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 mL/min and an injection volume of 2 μL was employed (split ratio of 10 : 1); Injector temperature 250^{o}C; Ion-source temperature 280^{o}C. The oven temperature was programmed from 110^{o}C (isothermal for 2 min.), with an increase of 10^{o}C/min, to 200^{o}C, then 5^{o}C/min to 280^{o}C, ending with a 9 min isothermal at 280^{o}C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass.

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the ethanol extract of *Aristolochia krysagathra*. These compounds were identified through mass spectrometry attached with GC. The compounds present in the ethanol extract of *Aristolochia krysagathra* identified by GC-MS analysis as shown in Fig. 1. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of *Aristolochia krysagathra* are presented in Table 1. The prevailing compounds in the ethanol extract were 3-O-Methyl-d-glucose (43.18%), 9, 12, 15-Octadecatrienric acid, methyl ester, (Z, Z, Z)-(11.46%), n-Hexadecanoic acid (10.25%), β-Sitosterol (9.53%), 3, 7, 11, 15-tetramethyl-2-hexadecan-1-ol (7.12%), Stigma sterol (4.83%), Phytol (4.46%), 9, 12, Octadecadienoicacid (Z, Z) (4.34%) and Octadecanoic acid (2.17%). Fig. 2, 3, 4 and 5 shows the mass spectra and structures of 3-O-Methyl-d-glucose, Hexadecanoic acid, ethyl ester, 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z) and Stigmasterol, respectively. Table 2 lists the major phytocompounds and their biological activities obtained through GC-MS study of *Aristolochia krysagathra*.

Table 1: Components detected in the whole plant ethanol extract of *Aristolochia krysagathra*

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	3.58	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene- [Synonyms: Pinocarveol]	$C_{10}H_{16}O$	152	0.84
2	3.79	Bicyclo[2.2.1]heptan-3-one, 6,6-dimethyl-2-methylene-	$C_{10}H_{14}O$	150	0.48
3	4.15	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl- [Synonyms: Myrtenol]	$C_{10}H_{16}O$	152	0.36
4	10.72	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	43.18
5	11.44	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	7.12
6	13.01	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	10.25
7	13.25	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.97
8	14.76	Phytol	$C_{20}H_{40}O$	296	4.46
9	15.18	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	4.34
10	15.27	9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	11.46
11	15.56	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.17
12	31.01	Stigmasterol	$C_{29}H_{48}O$	412	4.83
13	32.24	ß-Sitosterol	$C_{29}H_{50}O$	414	9.53

Table 2: Activity of phyto-components identified in the ethanol extract of whole plant of *Aristolochia krysagathra*

S. No.	Name of the compound	Molecular formula	Compound name	**Activity
1	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene- [Synonyms: Pinocarveol]	$C_{10}H_{16}O$	Alcoholic compound	Antimicrobial
2	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl- [Synonyms: Myrtenol]	$C_{10}H_{16}O$	Alcoholic compound	Antimicrobial
4	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	Sugar moiety	Preservative
5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Terpene alcohol	Antimicrobial antiinflammatory
6	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, lubricant, anti-androgenic, flavor, hemolytic 5-alpha reductase inhibitor

Cont…

S. No.	Name of the compound	Molecular formula	Compound name	**Activity
7	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	Palmitic acid ester	Antioxidant, hypocholesterolemic nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor
8	Phytol	$C_{20}H_{40}O$	Diterpene	Anticancer antioxidant anti-inflammatory diuretic
9	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	Linoleic acid	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide insectifuge, antihistaminic antieczemic, antiacne, 5-alpha reductase inhibitor antiandrogenic, antiarthritic, anticoronary, insectifuge
10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	Linolenic acid	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide insectifuge, antihistaminic antieczemic, antiacne, 5-alpha reductase inhibitor antiandrogenic, antiarthritic, anticoronary, insectifuge
11	Stigmasterol	$C_{29}H_{48}O$	Steroid	Antimicrobial antioxidant anti-inflammatory antiarthritic antiasthma diuretic
12	ß-Sitosterol	$C_{29}H_{50}O$	Steroid	Antimicrobial antioxidant anti-inflammatory antiarthritic antiasthma diuretic

**Source: Dr. Duke's: Phytochemical and Ethnobotanical Databases

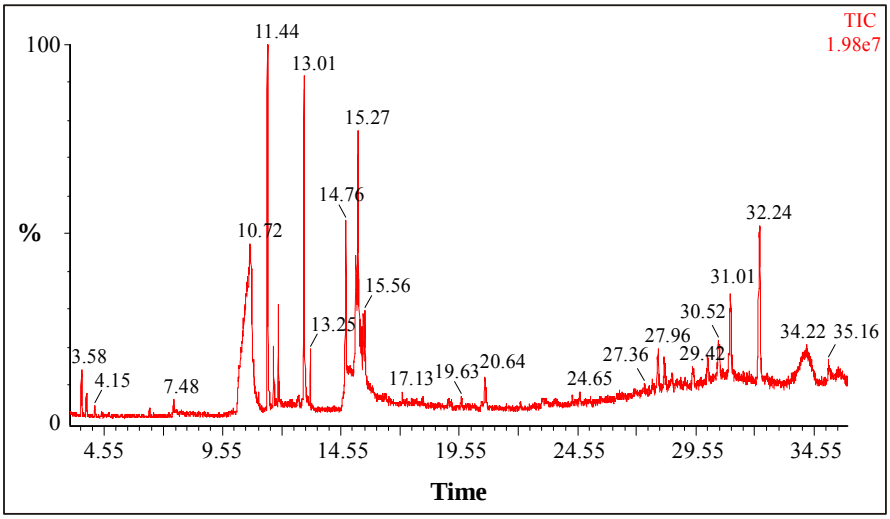

Fig. 1: GC-MS Chromotogram of the ethanol extract of whole plant of *Aristolochia krysagathra*

Among the identified phytochemicals, n-hexadecanoic acid has the property of antioxidant activity[13]. 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z) have the property of anti-inflammatory and antiarthritic as reported by earlier workers[14,15]. Omega-3-fatty acid have been found to be essential for normal growth and development and may play an important role in the prevention and treatment of coronay artery disease, hypertension, diabetes and arthritis, other inflammatory and autoimmune disorders and cancer[16-18]. Phytol is detected in *Aristolochia krysagathra* whole plant which was also found to be effective at different stages of the arthritis.

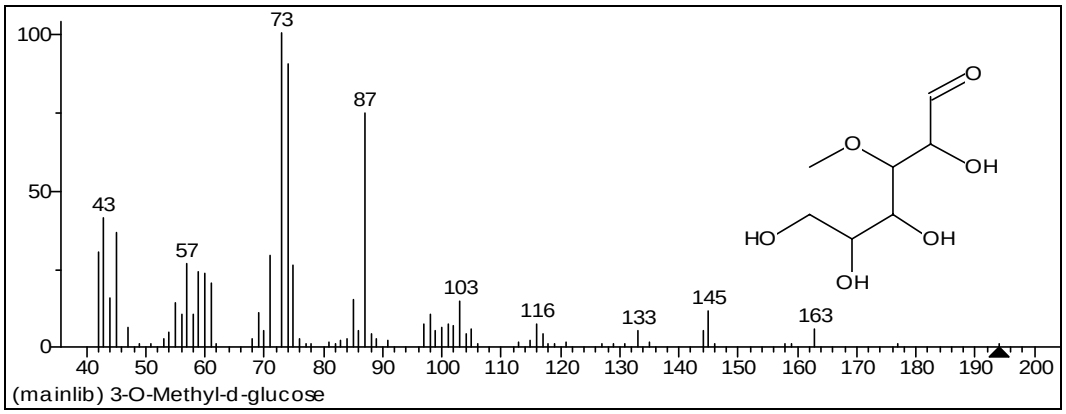

Fig. 2: Mass spectrum of 3-O-methyl-d-glucose

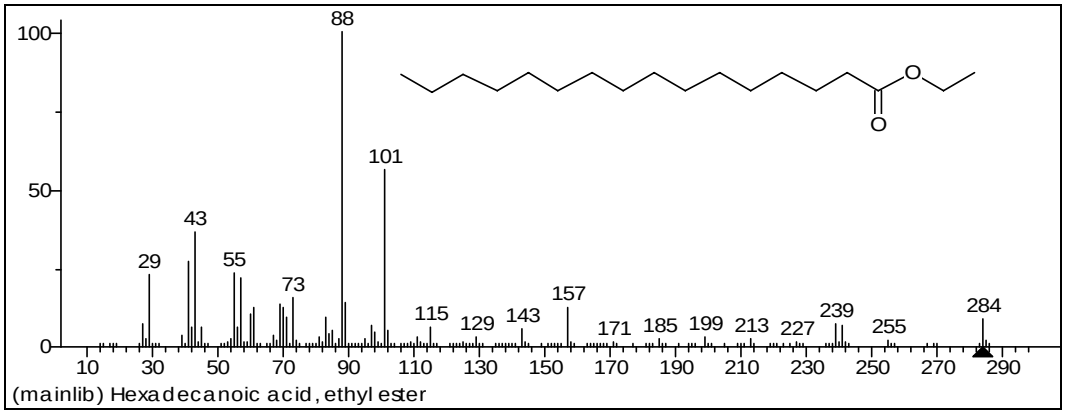

Fig. 3: Mass spectrum of hexadecanoic acid, ethyl ester

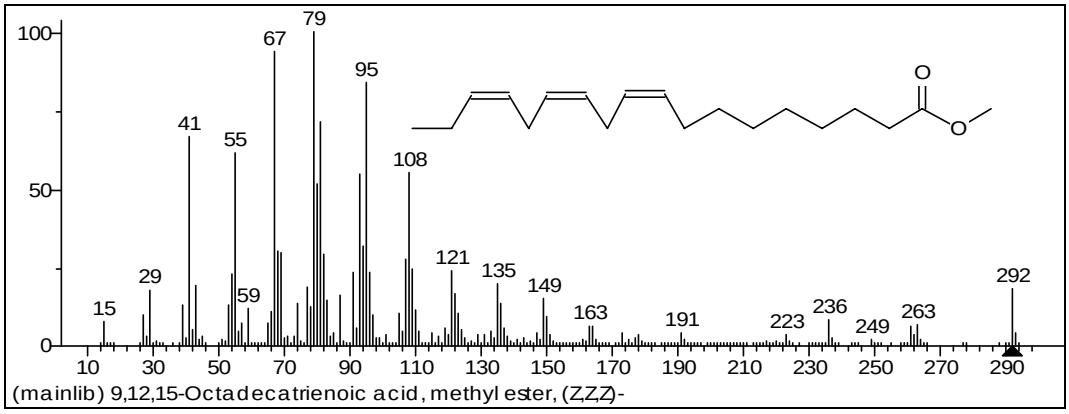

Fig. 4: Mass spectrum of 9, 12, 15-Octadecatrienoic acid, methyl ester (Z,Z,Z)

It was found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as phytol constitute a promosing novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases[19]. Stigmasterol is used as a precursor in the manufacture of semi synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of Vitamin D_3[20,21].

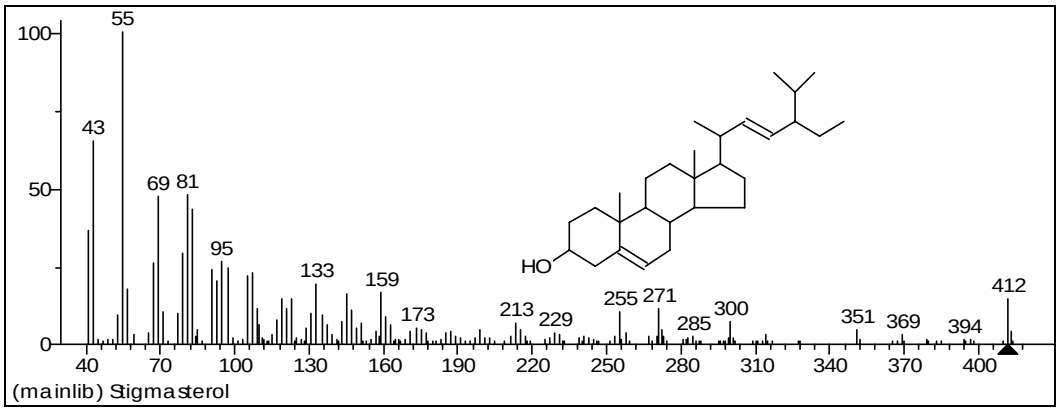

Fig. 5: Mass Spectrum of Stigmasterol

In the present study, 13 compounds were identified by Gas Chromatography-Mass Spectrometery (GC-MS) analysis. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practioners. However, isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, *Aristolochia krysagathra* contains various bioactive compound. So it is recommended as a plant of phytopharmaceutical importance.

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